



Effect of supplementation of biotin in total mixed ration of dairy cows on rumen fermentation characteristics by *in vitro* gas production technique



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Abstract

An experiment was conducted to assess the effect of supplementation of biotin on rumen fermentation parameters and microbial biomass production by *in vitro* gas production technique. Biotin was supplemented to the substrate - total mixed ration (TMR) at various doses viz., T1-0 (control), T2-0.5, T3-1.0, T4-1.5 and T5-2.0 mg/kg DM. The *in vitro* true dry matter degradability, total gas production, metabolizable energy and microbial protein production were found to be increased proportionally to the dose of biotin. However, the methane and volatile fatty acid production were not affected by biotin supplementation. Results revealed that the positive effects on *in vitro* ruminal fermentation were dose-dependent and biotin can be incorporated in the diet of dairy cows to improve nutrient digestibility and rumen biomass production.

Keywords: TMR, rumen fermentation characteristics, microbial biomass, biotin

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Biotin is an important water-soluble vitamin in the diet of both monogastric and ruminant animals. Biotin is naturally present in plants and also synthesized in the rumen by microbes. It plays an important role in a number of microbial metabolic processes, including gluconeogenesis and lipogenesis (Said, 2012). Dairy cows reared intensively may have a higher need for biotin. Various reports suggested that biotin synthesis was reduced by approximately 50 per cent when the ratio of concentrate to roughage increased. (Da Costa Gomez *et al.*, 1998). Similarly, increasing the concentrate portion also resulted in decreased ruminal biotin synthesis (Abel *et al.*, 2001). This suggests that high-concentrate diets fed to high-producing dairy cows can negatively influence net biotin synthesis in the rumen, due to the acidic conditions in the rumen, and this may aggravate the need for supplemental biotin. B complex vitamins are generally not supplemented to ruminants, due to the fact that rumen microbes have the ability to synthesise B complex vitamins in the rumen.

Rumen microbes convert inferior feed ingredients into high-quality microbial proteins to meet the nutritional needs of ruminants. This microbial protein supplies 60 to 85 % of amino acids reaching the animal's small intestine (Hristov, 2007; Owens *et al.*, 2009; Krizsan *et al.*, 2010). In the current scenario, measures to augment the efficiency of feed utilisation and production are essential to improve the economic viability of livestock farming. Feeding total mixed ration (TMR) will improve feed efficiency in animals because each mouthful of feed that the cow consumes contains balanced amount of nutrients. It will provide stable environment in the rumen for the activity of rumen microbes. It also gives energy and nitrogen sources at adequate level for optimum production of microbial protein and volatile fatty acids (Mackawa *et al.*, 2002). The supplementation dose of vitamin B for ruminants is not optimized. Hence, this experiment was intended to find the optimum dose of biotin for effective rumen biomass production.

Materials and methods

The *in vitro* gas production studies

were carried out using Hohenheim gas production technique (Menke and Steingass, 1988). The biotin was supplemented at different levels (T1, T2, T3, T4 and T5 - 0, 0.5, 1.0, 1.5 and 2.0 mg/kg DM respectively) of the basal diet. The basal diets comprised paddy straw and concentrate mixture in 29:71 ratio. Feed samples were analysed for proximate principles (AOAC, 2016). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method described by Van Soest *et al.* (1991).

To carry out *in vitro* gas production technique (IVGPT), rumen liquor was collected from early lactating crossbred cows fed on standard TMR in accordance with ICAR (2013) using stomach pump. The rumen liquor was strained through four layers of cheese cloth, transferred into pre-warmed CO₂-filled thermos flask. The temperature of the rumen fluid was maintained at 39°C throughout the preparation of the incubation medium. Fermentation was conducted in 100 ml glass syringe. The syringes were prewarmed (39°C) for 1-hour, before the addition of 30 ml of buffered rumen fluid into each syringe under CO₂ flushing. Three blank syringes containing only 30 ml of buffered rumen fluid were incubated to estimate gas production due to endogenous substrates for the blank corrections. 200 mg of TMRs were fortified with different levels of biotin and were incubated in 30 ml of incubation medium. The syringes were then placed in automatic shaker water bath incubator at 39°C. Analyses were completed in six replicates of each treatment and trial run in twice with readings of gas production recorded after incubation for 0 and 24 hours. The fermented fluid was collected for the estimation of volatile fatty acids, *in vitro* true dry matter degradability and *in vitro* true organic matter degradability (Banakar *et al.*, 2017). Metabolizable energy and microbial protein was calculated from the data as per Blummel and Lebzien (2001).

Total gas production

Gas produced (ml/ 200 mg substrate) by fermentation of substrate feed over a 24-hour period was measured after correcting corresponding blank values.

***In vitro* true DM and OM digestibility**

Goering and Van Soest (1970) method were followed for the determination of true dry matter digestibility (TDMD) and true organic matter digestibility (TOMD).

$$\text{TDMD \%} = \frac{(\text{DM taken for incubation} - \text{NDF residue}) \times 100}{(\text{DM taken for incubation})}$$

$$\text{TOMD \%} = \frac{(\text{OM taken for incubation} - \text{OM residue}) \times 100}{(\text{OM taken for incubation})}$$

Where, DM – Dry matter, OM – Organic matter, NDF – Neutral detergent fibre

Microbial biomass production

Microbial biomass production (MBP) of the TMR tested was calculated from TDOM using the equation

$$\text{MBP (mg)} = \text{TDOM (mg)} - (\text{Corrected gas production for 24 hrs} \times 2.20)$$

Where 2.20 is the stoichiometric factor for roughages (Blummel *et al.*, 1997) and for mixed diets (Blummel and Lebzién, 2001)

TDOM- Truly digested organic matter

Metabolizable energy (ME)

ME of target TMR was calculated by formula given by Menke and Stienkass (1988)

Table 1. Ingredient composition of total mixed ration used in *IVGPT*

Ingredient	Quantity (parts per quintal)
Maize	23
Rice polish	10
Corn gluten fibre	10
De-oiled rice bran	10
Alfalfa pellet	8
Coconut oil cake	8
Paddy straw	29
Mineral mixture	1
Salt	1
Total	100

$$\text{ME (KJ/kg DM)} = 1.24 + 0.146 \times \text{gas (ml / 200mg DM)} + 0.007 \times \text{CP} + 0.0224 \times \text{EE}$$

Where, CP - Crude protein, EE - Ether extract

Methane estimation

The percentage of methane production was estimated by collecting and injecting the gas produced on *in vitro* study into the Methane Gas Analyser (0-100%; Precision Equipment Private Limited) at Central Instrumentation Laboratory, CVAS, Mannuthy. (Purushothaman *et al.*, 2019; Sadan *et al.*, 2019 and Neenu, 2021).

Estimation of volatile fatty acids

Volatile fatty acid composition of the inoculum was estimated using 7890A NUCON 5700 gas chromatograph, as per standard procedure described by Filipek and Dvorak (2009). On completion of the incubation, the buffered rumen liquor was filtered through four layers of muslin cloth and approximately 0.8 mL of the sample was preserved with 200 µl of 25 per cent metaphosphoric acid and allowed to stand for half an hour, then centrifuged at 7000 rpm for 20 min at 4°C. The samples preserved in this way were immediately analysed or stored at -20°C for future analysis.

Observations made on the various parameters viz. true dry matter digestibility

Table 2. Chemical composition of TMR¹ used in *IVGPT* (% DM basis)

Parameter	Nutrient composition (%)
Dry matter	92.63 ± 0.41
Crude protein	13.28 ± 0.12
Ether extract	3.74 ± 0.10
Crude fibre	12.55 ± 0.12
Total ash	11.45 ± 0.12
Nitrogen free extract	58.99 ± 0.27
Acid insoluble ash	5.11 ± 0.1
Calcium	0.90 ± 0.02
Phosphorus	0.56 ± 0.02
Neutral detergent fibre	35.25 ± 0.19
Acid detergent fibre	24.74 ± 0.24

¹Mean values are based on six replicates with S.E.

Table 3. *In vitro* gas production and fermentation parameters of TMR supplemented with biotin in cross bred cows

Treatments	Total gas (mL)	CH ₄ (%)	ME (MJ/kg DM)	TDMD (%)	TOMD (%)	MBP (mg)
T1	28.54± 0.35 ^a	19.25 ± 0.23	5.61 ± 0.05 ^a	67.39 ± 0.16 ^a	70.43 ± 0.55 ^a	63.36 ± 1.52 ^a
T2	30.25± 0.18 ^b	19.08± 0.15	5.86 ± 0.03 ^b	73.2 ± 0.31 ^b	74.96 ± 0.72 ^b	67.55 ± 1.38 ^b
T3	33.02± 0.20 ^c	18.84± 0.25	6.27 ± 0.03 ^c	75.28 ± 0.3 ^c	79.65 ± 0.25 ^c	69.86 ± 0.66 ^{bc}
T4	35.23± 0.25 ^d	18.72± 0.19	6.59 ± 0.04 ^d	77.42 ± 0.27 ^d	83.27 ± 0.48 ^e	71.59 ± 0.98 ^c
T5	35.93± 0.20 ^d	18.80± 0.34	6.7 ± 0.04 ^d	77.86 ± 0.21 ^d	81.4 ± 0.35 ^d	66.88 ± 0.97 ^b
F-value	159.53	0.822	159.53	273.86	110.34	7.49
P-value	0.001**	0.523 ^{ns}	0.001**	0.001**	0.001**	0.001**

¹Mean values are based on six replicates with S.E.

**Mean± S.E. of different treatment having different alphabets as superscripts within a column differ significantly at p<0.01

NS- Non-Significant

Table 4. Volatile fatty acid concentration¹ of TMR supplemented with biotin in cross bred cows assessed *in vitro*

Treatments	Acetic acid (mMol/L)	Propionic acid (mMol/L)	Butyric acid (mMol/L)	Total volatile fatty acids (mMol/L)	Acetate: propionate ratio
T1	46.77±0.29	19.04±0.52	5.28±0.16	71.09±0.30	2.47±0.08
T2	46.12±0.74	19.08±0.58	6.16±0.10	71.36±0.97	2.43±0.08
T3	46.71±0.41	19.94±0.57	5.42±0.21	72.08±0.65	2.35±0.07
T4	45.94±0.26	20.59±0.51	5.73±0.29	72.26±0.89	2.24±0.05
T5	45.91±0.16	19.77±0.45	5.68±0.51	71.36±0.25	2.33±0.05
F- value	1.024	1.484	1.341	0.563	1.692
P- value	0.414 ^{ns}	0.237 ^{ns}	0.282 ^{ns}	0.691 ^{ns}	0.183 ^{ns}

¹Mean values are based on six replicates with S.E.

NS – Non-Significant

(TDMD) and true organic matter digestibility (TOMD), microbial biomass production, metabolizable energy and methane production were subjected to cluster analysis and based on this, the best level of biotin was identified.

Data gathered on the various parameters were analysed statistically as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique, using the statistical software, SPSS - version 24.0 (IBM Corp., 2016)

Results and discussion

Proximate composition

The chemical composition and fiber fractions of the evaluated samples are

presented in Table 2. The CP, EE, CF, total ash, NFE, AIA, calcium, phosphorus, NDF and ADF contents of the basal substrate were found to be 13.28±0.12, 3.74±0.10, 12.55±0.12, 11.45±0.12, 58.99±0.27, 5.11±0.1, 0.90±0.02, 0.56±0.02, 35.25±0.19 and 24.74±0.24, on DM basis, respectively.

In vitro gas production

Ruminal fermentability characteristics evaluated by *in vitro* gas production are depicted in Table 3. In biotin supplemented TMRs the gas production for 24 hours ranged from 28.54±0.35 to 35.93±0.20 mL. Biotin supplementation significantly increased the amount of gas produced during the first twenty-four hours. Methane varied from 18.72 ± 0.19 to 19.25 ± 0.23 %, which was found

to be similar among the groups. Contrary to the present findings, Poolthajit *et al.* (2021) reported increased methane production in TMR supplemented with combination of betaine, biotin and chromium picolinate @3 g/kg DM and 6 g/kg DM, but it had no effect at high levels - 9 g/kg DM assessed by IVGPT.

Digestibility and metabolizable energy

Metabolisable energy values ranged from 5.61 ± 0.05 to 6.7 ± 0.04 . The corresponding TDMD % ranged from 67.39 ± 0.16 to 77.86 ± 0.21 , the TOMD % from 70.43 ± 0.55 to 81.4 ± 0.35 . Supplementation of biotin to basal diet significantly ($P < 0.01$) improved ME, the *in vitro* TDMD and TOMD (Table 3). Higher level of supplementation showed significantly higher *in vitro* TDMD and TOMD (biotin 1.5 mg/kg DM level). Increase in nutrient digestibility due to supplementation may be because of the stimulation of rumen microbial growth.

In accordance with present results, Cruyvagen and Bunge (2004) also reported an improved fermentability and NDF digestibility in cows which were supplemented with biotin. Kandathil and Bandla (2019) reported that fortification of the basal substrate with biotin increased the total gas production in Deoni cows. They reported that B-vitamin synthesised by one microbe is consumed by others. As there is no absorption of B vitamins in rumen, the supplementation of B vitamins through feed could augment rumen biomass production. On the contrary, Grewal *et al.* (2016) reported that supplementation of biotin @ 1.33 mg/kg DM of complete feed had no effect on the net gas production, digestibility of true OM, NDF and ME availability.

Microbial biomass production

The MBP (mg/200mg DM) calculated from the gas production and TOMD data are listed in Table 3. MBP (mg) production ranged from 63.36 ± 1.52 to 71.59 ± 0.98 respectively. The statistical analysis of the data on MBP (mg/200mg DM) revealed a significant difference ($P < 0.01$) among the treatments. Microbial nitrogen is the major source of protein for ruminants and is utilized to meet the maintenance requirement of the animal. It was

highest in T4 and is indicative of a good amount of fermentable substrate aiding the growth and development of rumen microorganism. Gas production, ME, TDMD and TOMD was highest in T4 and T5, whereas MBP was highest in T4.

Biotin supplementation in this trial possibly stimulated cellulolytic bacteria as they depend on other microbes for their metabolic biotin requirements (Baldwin and Allison, 1983). Where biotin was omitted from an *in vitro* medium, Milligan *et al.* (1967) observed a decrease in fibre digestion. They postulated that the biotin deficiency caused a blockage of one or more steps in the propionate production pathway resulting in a depletion of vital intermediates, which in turn resulted in reduced rates of cellulose digestion.

Volatile fatty acids production

Data related to total volatile fatty acids (TVFA; mmol/L), acetic acid, propionic acid, butyric acid and acetate: propionate ratio is presented in Table 4. Statistical analysis of the data revealed that there was no significant difference ($P > 0.05$) in the TVFA and individual fatty acids among the groups. Similarly, Zimmerly and Weiss (2001) reported that supplementation of dietary biotin (0, 10, or 20 mg/day) had no effect on the molar percentages of the ruminal volatile fatty acids Holstein cows. Suksombat *et al.* (2011) also reported that incorporating biotin supplements at the rate of 0, 20 and 40 mg/cow/day in Holstein Friesian dairy cows had similar concentration of volatile fatty acids among the groups.

Conclusion

The study revealed that there was major variation in *in vitro* rumen fermentation parameters with addition of biotin. Supplementation of biotin for manipulating rumen fermentation was effective and showed positive results on *in vitro* true dry matter degradability, *in vitro* organic matter digestibility, ME and microbial biomass production. It could be inferred that biotin supplementation @ 1.5 mg/kg DM can be used for optimizing the rumen fermentation characteristics in dairy cows. In future *in vivo* research to be done to validate the results.

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Conflict of interest

The authors declare that they have no conflict of interest.

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